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Carob Bean Gum (Clarified)

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CAROB BEAN GUM (CLARIFIED)

Prepared at the 82nd JECFA and published in JECFA Monograph 19 (2016) superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 25th JECFA (1981) for carob bean gum.

SYNONYMS

Locust bean gum clarified, INS No. 410

DEFINITION

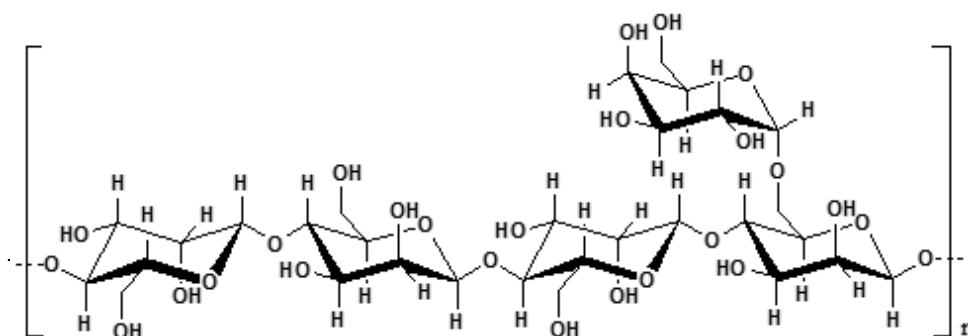
Carob bean gum, also known as locust bean gum, is a galactomannan polysaccharide obtained from the seeds of *Ceratonia siliqua* (L.) Taub. (Fam. *Leguminosae*). The ground endosperm consists mainly of high molecular weight (approximately 50,000-3,000,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 4:1. The seeds are dehusked by treating the seeds with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ, followed by milling and screening of the endosperm to obtain native carob bean gum.

The gum is clarified by dispersing in hot water, filtration and precipitation with ethanol or isopropanol, filtering, drying and milling. The clarified carob bean gum does not contain cell wall materials. Clarified carob bean gum in the market is normally standardized with sugars for viscosity and reactivity.

C.A.S. number

9000-40-2

Structural formula



DESCRIPTION

White to yellowish white, nearly odourless powder

FUNCTIONAL USES

Stabilizer, thickener, emulsifier, gelling agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in ethanol

Gel formation

Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.

Viscosity

Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the gum is completely and uniformly dissolved. An opalescent, slightly

viscous solution is formed. Transfer 100 ml of this solution into another 400-ml beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).

Gum constituents
(Vol. 4)

Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.

PURITY

Loss on drying (Vol. 4)

Not more than 14% (105°, 5 h)

Total ash (Vol. 4)

Not more than 1.2% (800°, 3-4 h)

Acid-insoluble matter
(Vol. 4)

Not more than 3.5%

Protein (Vol. 4)

Not more than 1.0%

Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under "General Methods, Inorganic components"). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.

Starch

To a 1 in 10 solution of the sample add a few drops of iodine TS; no blue colour is produced

Residual solvents

Not more than 1% of ethanol or isopropanol, singly or in combination
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

Not more than 3 mg/kg

Arsenic (Vol. 4)

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria
(Vol. 4)

Initially prepare a 10⁻¹ dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender.

Total (aerobic) plate count: Not more than 5,000 CFU/g

E. coli: Negative in 1 g

Salmonella: Negative in 25 g

Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Method I)

Internal standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 μ l of 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

Standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 μ l ethanol and weigh to within 0.01mg. Inject 15 μ l isopropanol through the septum and reweigh the vial.

Blank solution: Add 5.0 ml of water and pipette 1.0 ml of the internal standard solution into a headspace vial. Seal the vial and mix the contents using a vortex mixer.

Calibration solution: Add 4.0 ml of water into the headspace vial. Pipette 1.0 ml each of the internal standard solution and the standard solution. Seal the vial and mix the contents using a vortex mixer.

Preparation of sample: Accurately weigh 0.500 ± 0.001 g of sample in a small weighing boat. Pipette 5 ml of water and 1 ml internal standard solution into a headspace vial. Add the sample carefully to prevent clumping of sample at the bottom of the vial. Seal the vial and mix the contents using a vortex mixer. Do not shake the sample vial.

Follow the procedure described in Vol. 4.

CASSIA GUM (TENTATIVE)

Revised and made tentative at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010). An ADI "not specified" was established at the 71st JECFA (2009).

Information required:

- Suitable method for the determination of anthraquinones, including:
 - the use of standard (reference) materials
 - the extraction efficiency of the initial steps
 - the recovery of the analytes in question
 - performance data for the method
 - results of analysis by an accredited laboratory of a minimum of five batches of material

The tentative specifications will be withdrawn unless the requested information is submitted before 31st December 2017.

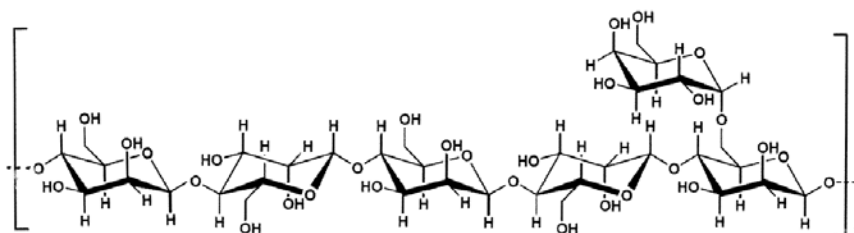
SYNONYMS

INS 427

DEFINITION

Primarily the ground purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia*, (Fam. *Leguminales*) containing less than 0.05% of *Cassia occidentalis*. It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 5:1. The structural formula for cassia gum galactomannan is given below. The seeds are dehusked and degermed by thermal mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is further purified by extraction with isopropanol.

Structural formula



Assay

Not less than 75% of galactomannans

DESCRIPTION

Pale yellow to off-white, odourless free-flowing powder

FUNCTIONAL USES

Thickener, emulsifier, foam stabilizer, moisture retention agent and texturizing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility

Insoluble in ethanol

Disperses well in cold water forming colloidal solutions.

Gel formation with borate

Add sufficient amounts of sodium borate TS to an aqueous dispersion of the sample sufficient to raise the pH to above 9; a gel is formed.

<u>Gel formation with xanthan gum</u>	Passes test See description under tests
<u>Gum constituents</u> (Vol. 4)	Proceed as directed under Gum Constituents Identification (Vol. 4) using 100 mg of sample instead of 200 mg and 1-10 µl of the hydrolysate instead of 1-5 µl. Use galactose and mannose as reference standards. These constituents should be present.
<u>Viscosity</u>	Less than 500 mPas (25°, 2h) (1% solution) See description under TESTS
<u>pH</u> (Vol. 4)	5.5-8.0 (1%)
PURITY	
<u>Loss on drying</u> (Vol. 4)	Not more than 12% (105°, 5 h)
<u>Total ash</u> (Vol. 4)	Not more than 1.2%
<u>Acid-insoluble matter</u> (Vol. 4)	Not more than 2.0%
<u>Protein</u> (Vol. 4)	Not more than 7.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method; Vol. 4). The percent of nitrogen in the sample multiplied by 6.25 gives the percent of protein in the sample.
<u>Crude fat</u>	Not more than 1% See description under TESTS
<u>Starch</u>	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
<u>Anthraquinones</u>	Not more than 0.5 mg/kg See description under TESTS
<u>Residual solvents</u> (Vol. 4)	Isopropanol: Not more than 1.0% See description under Tests
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Microbiological criteria</u> (Vol. 4)	Total plate count: Not more than 5,000 cfu/g Yeast and mould: Not more than 100 cfu/g <i>E. coli</i> : Negative in 1 g <i>Salmonella</i> : Negative in 25 g

TESTS**IDENTIFICATION TESTS**

<u>Gel formation with xanthan gum</u>	Weigh 1.5 g of the sample and 1.5 g of xanthan gum and blend them. Add this blend with (rapid stirring) into 300 ml water at 80° in a 400 ml
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beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60° during the stirring process). Discontinue stirring and allow the mixture to cool at room temperature for at least 2 h.

A firm, viscoelastic gel forms after the temperature drops below 40°, but no such gel forms in a 1% control solution of cassia gum or xanthan gum alone prepared in a similar manner.

Viscosity

Weigh 5 g of the sample in a plastic dish and 495 g of distilled water at 20° in a 1000 ml beaker. Add a magnetic bar and place the beaker on the agitation plate. Adjust the speed of agitation to 750 rpm. Introduce quickly the 5 g of sample in the water and cover the beaker with a watch glass. Keep the temperature at 90° for 15 min. Cool the solution at 25° (the cooling must be $\pm 1.5^\circ$) in a water bath and measure the viscosity after 2 h at 25° using a RVT Brookfield Spindle 1, speed 20 rpm. Repeat the procedure with a sample of 5 g of carob (locust) bean gum.

(Note: The viscosity of the cassia gum (150 - 500 mPas) must be less than 50% that of carob bean gum (2000 - 3500 mPas))

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Vol. 4; Method I)

Internal standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 μ l of 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

Standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 μ l ethanol and weigh to within 0.01 mg. Inject 15 μ l isopropanol through the septum and reweigh the vial.

Blank solution: Add 5.0 ml of water and pipette 1.0 ml of the internal standard solution into a headspace vial. Seal the vial and mix the contents using a vortex mixer.

Calibration solution: Add 4.0 ml of water into the headspace vial. Pipette 1.0 ml each of the internal standard solution and the standard solution. Seal the vial and mix the contents using a vortex mixer.

Preparation of sample: Accurately weigh 0.500 ± 0.001 g of sample in a small weighing boat. Pipette 5 ml of water and 1 ml internal standard solution into a headspace vial. Add the sample carefully to prevent clumping of sample at the bottom of the vial. Seal the vial and mix the contents using a vortex mixer. Do not shake the sample vial.

Follow the procedure described in Vol. 4.

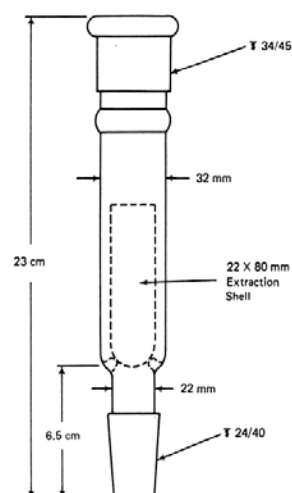
Crude fat

Apparatus

The apparatus consisting of a Butt-type extractor, as shown below, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-ml Erlenmeyer flask.

Procedure

Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a 15-cm filter paper, roll the paper tightly around the sample, and place it in a suitable extraction shell. Plug the top of the shell with cotton previously extracted with hexane, and place the shell in the extractor. Attach the extractor to a dry 125-ml Erlenmeyer flask containing about 50 ml of hexane and to a water-cooled condenser, apply heat to the flask to produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few ml of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odor of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.



Butt-Type Extractor for fat determination.

NOTE: The method for crude fat is referenced from the Food Chemicals Codex, 6th Edition, 2008, p. 1163. Reprinted with permission from the US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

Anthraquinones

A validated method for determination of anthraquinones at the specified maximum level is requested

METHOD OF ASSAY The difference between 100 and the sum of the percent Loss on Drying, Total Ash, Acid-Insoluble Matter, Protein and Crude Fat represents the percent *Galactomannans*.